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Syntheses of C-13 and C-14-labeled versions of the investigational proteasome inhibitor MLN9708†

Mihaela Plesescu,* Eric L. Elliott, Yuexian Li, and Shimoga R. Prakash

MLN9708 (ixazomib citrate) is an investigational, orally bioavailable proteasome inhibitor that is under development by Millennium in clinical studies in both hematologic and nonhematologic malignancies. The stable isotope-labeled MLN9708 was required for bio-analytical studies. [$^{13}C_9$]-MLN9708 (11) was synthesized in seven steps from the uniformly labeled [$^{13}C_6$]-1,4-dichlorobenzene (3) and [1-^{13}C]-acetyl chloride. Because of the presence of two chlorine atoms and a boron atom, compound 6 was further reacted with [$^{13}C_2$]-glycine to provide an internal standard that is well separated from the parent compound during mass spectrometric analysis. The radiolabeled version was prepared to support metabolite profiling and whole body autoradiography studies in experimental animals. [^{14}C]-MLN9708 (19) was synthesized in six steps from commercially available [^{14}C]-barium carbonate. The key intermediate, [carboxyl-^{14}C]-2,5-dichlorobenzoic acid (14), was prepared by selective lithiation of 1-bromo-2,5-dichlorobenzene (12) followed by carbonation with [^{14}C]-barium carbonate. In preparation for a one-time human absorption, distribution, metabolism and excretion (ADME) study, the stability of [^{14}C]-MLN9708 and its precursors were also evaluated. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: [$^{13}C_9$]-2,5-dichlorohippuric acid; [$^{13}C_9$]-MLN9708; [carboxyl-^{14}C]-2,5-dichlorobenzoic acid; [^{14}C]-MLN9708

Introduction

MLN9708 (**1**, 4-(*R*,*S*)-(carboxymethyl)-2-((*R*)-1-(2-(2,5-dichlorobenzamido)acetamido)-3-methylbutyl)-6-oxo-1,3,2-dioxaborinane-4-carboxylic acid, Figure 1), is a potent, reversible and selective inhibitor of the 20S proteasome.[1,2] The proteasome is an attractive target for developing small molecule inhibitors for cancer therapies as demonstrated by VELCADE® (Cambridge, MA, USA) (bortezomib) for Injection.[3,4] In the aqueous media, MLN9708 rapidly hydrolyzes to MLN2238 (**2**, Figure 1), the biologically active form.[5] The stable isotope-labeled version of MLN9708 (**11**) was required as an internal standard for mass spectrometry-based bio-analytical assays. MLN9708 (with the chemical formula $C_{20}H_{23}BCl_2N_2O_9$) contains two chlorine atoms and a boron atom; thus, a labeled version that has at least 8 amu higher than the unlabeled version is required to completely separate the labeled molecular ion clusters from the unlabeled clusters during mass spectrometric assays. Labeling with carbon-14 was also required to address *in vivo* disposition of MLN9708.

Results and discussion

Synthesis of [$^{13}C_9$]-MLN9708 (11)

The synthesis of [$^{13}C_9$]-MLN9708 commenced from commercially available [$^{13}C_6$]-1,4-dichlorobenzene (**3**), which was treated with a slight excess of [1-^{13}C]-acetyl chloride under Friedel-Crafts acylation conditions.[6] The desired ketone (**4**) was subjected next to the haloform reaction to produce the uniformly labeled carboxylic acid (**5**). Subsequent activation of the acid with thionyl chloride and coupling with [$^{13}C_2$]-glycine via a Schotten-Baumann type amide synthesis provided the [$^{13}C_9$]-dichlorohippuric acid (**7**).[7] The crude acid was recrystallized from water at 100 °C to obtain higher chemical purity (99%). The (1*S*,2*S*,3*R*,5*S*)-pinanediol leucine boronate **8** was elaborated from commercially available isobutyl boronic acid and (+)-pinanediol in five steps.[8] Peptide coupling of **8** with the acid **7** provided the protected boronate of MLN9708 (**9**).[9] Deprotection of the boronic acid was accomplished via transesterification with isobutylboronic acid. After the work-up, [$^{13}C_9$]-MLN2238 (**10**) was isolated by precipitation from dichloromethane using heptane. In the last step, [$^{13}C_9$]-MLN2238 was subjected to esterification with citric acid at a temperature of 60 °C. Upon cooling to ambient temperature, [$^{13}C_9$]-MLN9708 (**11**) was isolated as a solid.[7]

The protonated molecular ion $[M+H]^+$ of MLN2238 is labile and undergoes in-source dehydration (−18 Da) to yield an observed $[M\text{-}H_2O]^+$ at m/z 343 (Figure 2). The same pattern was maintained for the internal standard, [$^{13}C_9$]-MLN9708. MLN2238 presents as a

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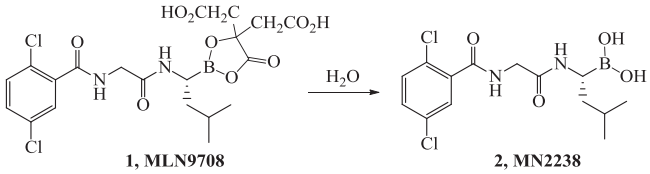


Figure 1. Structures of MLN9708 and MLN2238.

trimeric anhydride in aprotic media, and its dimer and trimer forms are also detected during LC–MS analysis.

Synthesis of [^{14}C]-MLN9708 (19)

The peptide structure of MLN2238 (the free acid of MLN9708) provides two potential sites for the radiolabeling, either on the 2,5-dichlorobenzoic acid or the glycine half (Figure 3).

In choosing the adequate starting material, we reviewed the previously prepared carbonyl-labeled boronic acids, [^{14}C]-MLN0273[10] and [^{14}C]-bortezomib[11] (Figure 4).

Furthermore, the radiolabeling of MLN9708 was designed to incorporate the carbon-14 label on a metabolically stable position. On the basis of the known metabolism of bortezomib (oxidative deboronation, hydroxylation on the right-hand side of the molecule),[12] we chose to label the carbonyl moiety adjacent to the 2,5-dichlorophenyl group.

The key precursor required for the labeling step, [carboxyl-^{14}C]-2,5-dichlorobenzoic acid (**14**), was prepared from 1-bromo-2,5-dichlorobenzene (**12**), which was selectively lithiated at very low temperature to form **13** in solution. The carbonation reaction of **13** was conducted at −78 °C using a stream of $^{14}CO_2$ freshly prepared from commercially available [^{14}C]-barium carbonate treated with concentrated sulfuric acid.[13] The desired [^{14}C]-2,5-dichlorobenzoic acid **14** was isolated from the reaction mixture

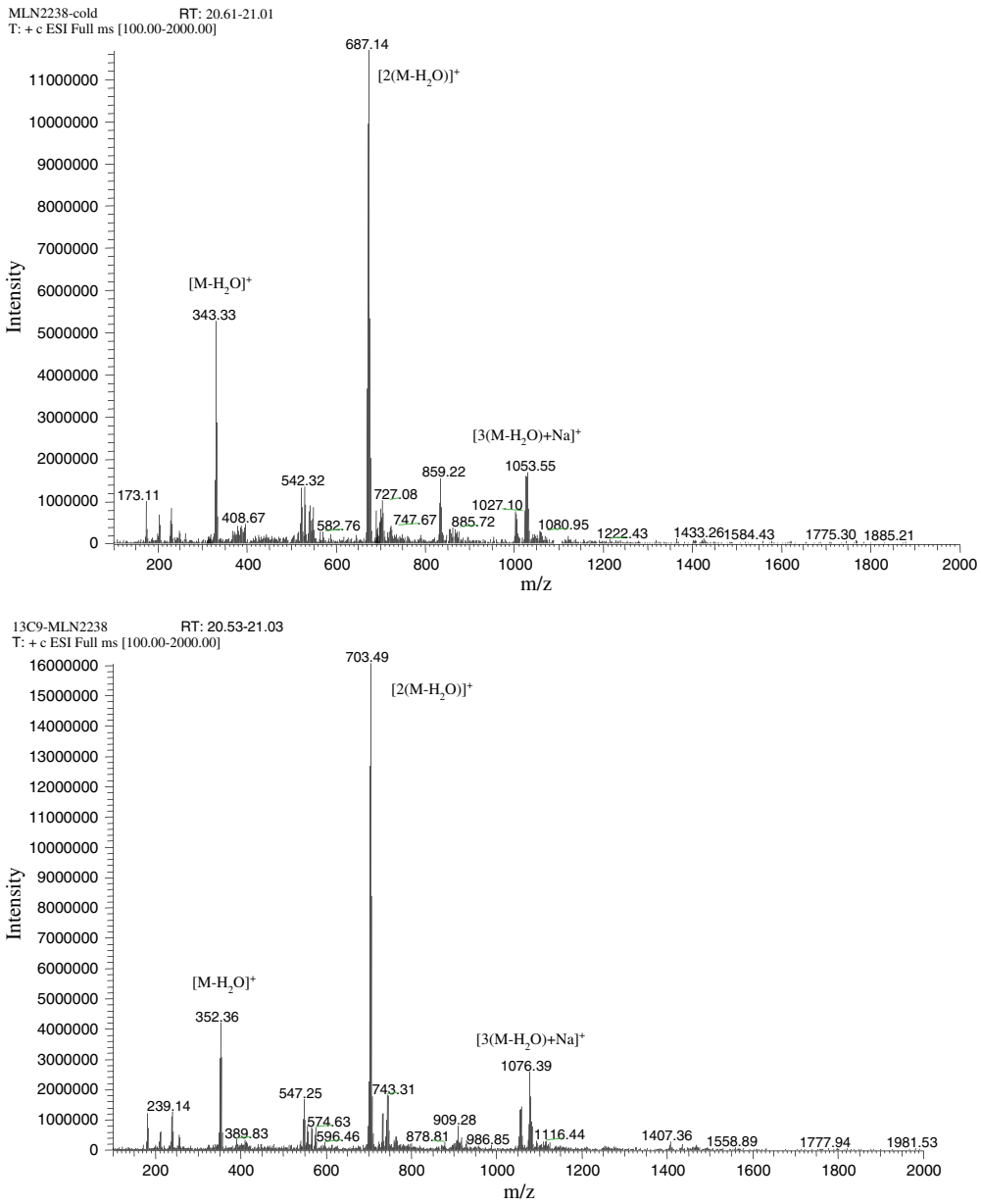


Figure 2. ESI–MS spectra of MLN2238 (MW = 361) and [$^{13}C_9$]-MLN2238 (MW = 370).

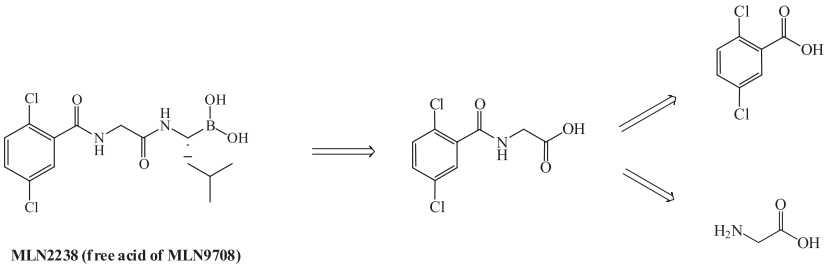


Figure 3. Strategy for labeling [^{14}C]-MLN9708.

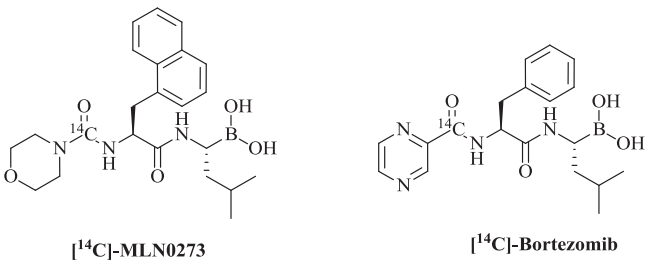


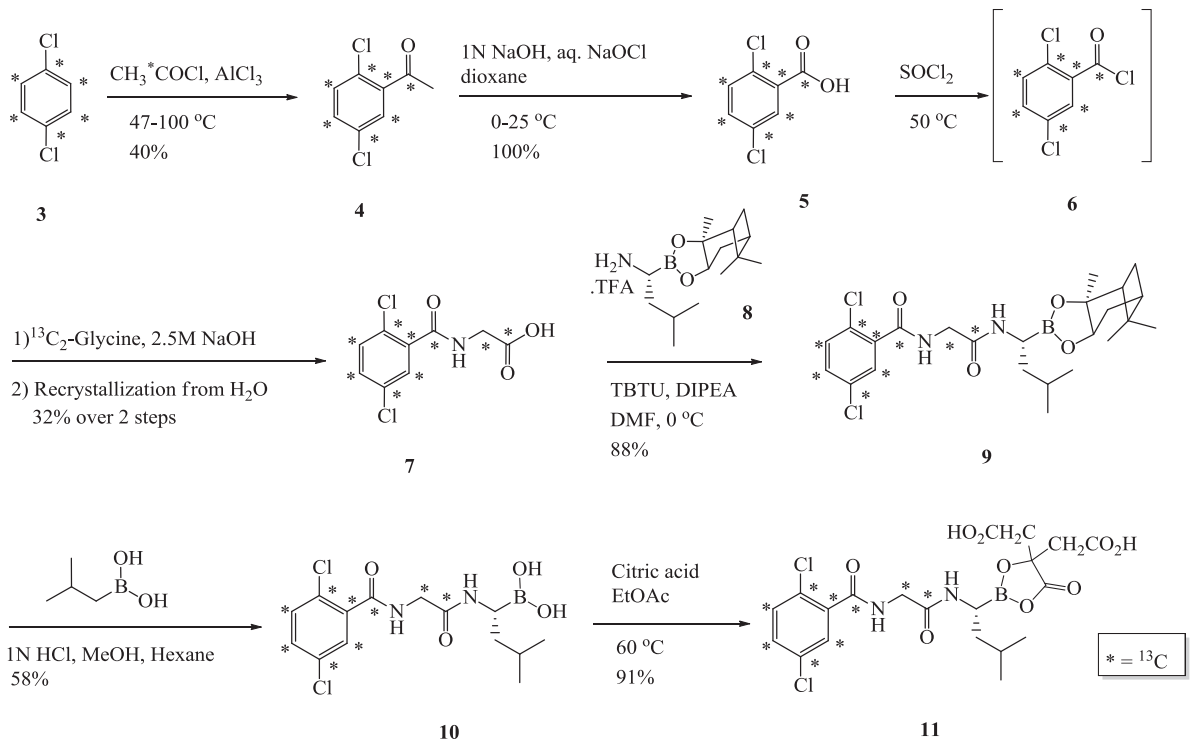
Figure 4. Structures of [^{14}C]-MLN0273 and [^{14}C]-Bortezomib.

and coupled with glycine methyl ester to form **15**. Because of the small scale, this reaction was preferred over the treatment of the acid with thionyl chloride and the subsequent coupling with glycine. Instead, the ester **15** was saponified to give carboxylic acid **16**, which was recrystallized from hot water as previously described in the synthesis of the stable isotope version. The next three steps were similar to the steps shown in Scheme 1 (compounds **7–11**).[7,9] Unfortunately, after the transesterification step, significant radiochemical impurities were formed in the presence of oxygen because of the loss of the boronic acid moiety. The pure [^{14}C]-MLN2238 (**18**) was isolated after the reversed phase chromatographic purification.

In support of clinical investigations in patients, a one-time, small-scale clinical absorption, distribution, metabolism and excretion study is being planned with [^{14}C]-MLN9708 (**19**). As shown in Table 1, at high specific activity, the radiolabeled MLN9708 is very unstable when stored at −80 °C over a prolonged period. A second batch showed radiochemical decomposition of nearly 1% per week. Because of this fast radiochemical decomposition, the material would not meet impurity specifications before use in the clinic.

It is not possible to blend at the penultimate step ([^{14}C]-MLN2238 with unlabeled MLN2238) because of the poor stability of the isolated labeled material. [^{14}C]-MLN2238 (**18**) at high specific activity is very unstable; radiochemical decomposition of 1–2% was observed during drying under vacuum/lyophilization overnight at room temperature.

The only viable way to make stable [^{14}C]-MLN9708 with desired lower specific activity was by the use of the [^{14}C]-dichlorohippuric acid (**16**) step for blending labeled and unlabeled material (Scheme 2, compounds **16–19**).



Scheme 1. Synthesis of [$^{13}C_9$]-MLN9708 (11).

Preliminary stability assessment indicated that the blended [^{14}C]-MLN9708 material (specific activity 16.6 μCi/mg) was stable for over 3 months when stored at −80 °C (Table 2).

Stability studies showed that high specific activity [^{14}C]-2,5-dichlorohippuric acid (**16**, 53 mCi/mmol) can be stored at −80 °C for long periods (Table 3). This material can be successfully recrystallized from water and acetone to generate the pure product that may be subsequently utilized to prepare a new batch of [^{14}C]-MLN9708.

Conclusion

A stable isotope-labeled version of MLN9708 (**11**) was prepared in 6% overall yield for use as an internal standard in bio-analytical assays. In support of absorption, distribution, metabolism and excretion studies conducted in laboratory animals, we synthesized [^{14}C]-MLN9708 (**19**) by using [^{14}C]-barium carbonate as the starting material. The synthesis was completed in five steps with an overall radiochemical yield of 7.3%. In preparation for a one-dose study in patients with radiolabeled MLN9708, we investigated the stability of the compound at different specific activities and of an intermediate stored at −80 °C. The stability studies showed

Table 1. Stability data of [^{14}C]-MLN9708 at high specific activities (stored at −80 °C)

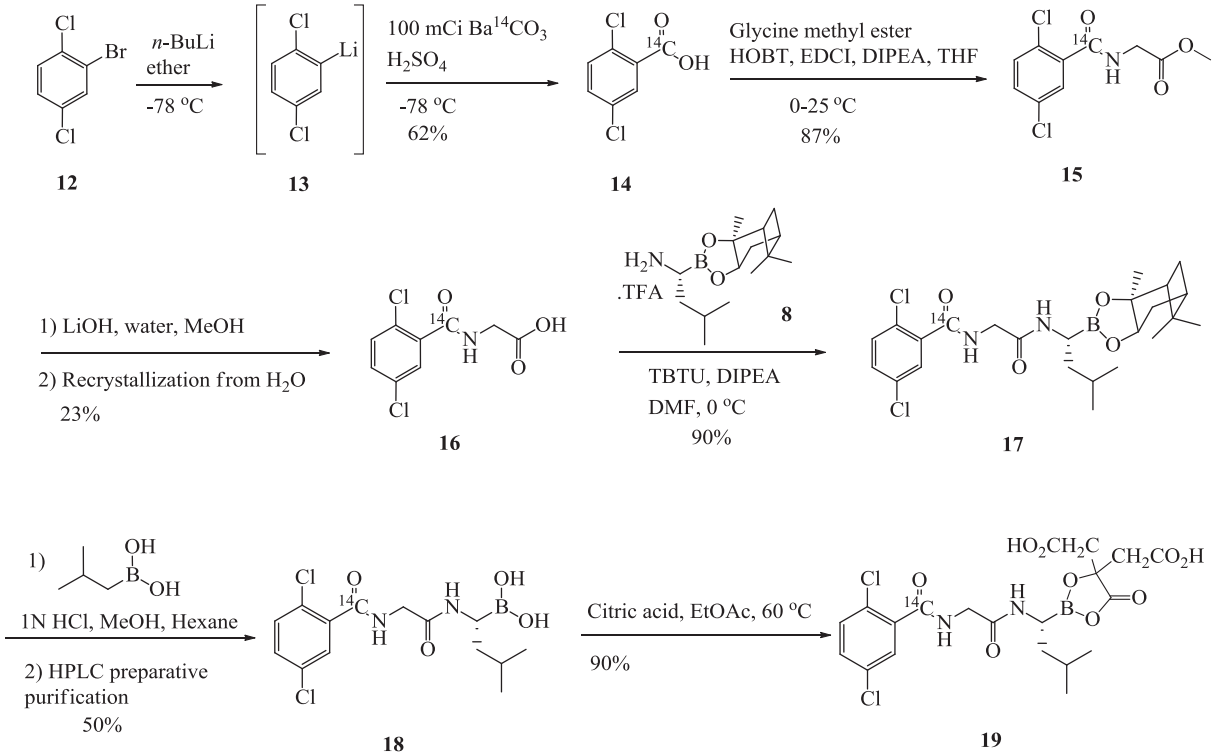
Time point	Radiochemical purity, %	Largest individual radiochemical impurity, %
First batch of [^{14}C]-MLN9708 (specific activity: 108.8 μCi/mg)		
Initial	98.6	1.0
16 months	70.6	5.8
42 months	37.7	12.3
Second batch of [^{14}C]-MLN9708 (specific activity: 112.25 μCi/mg)		
Initial	98.7	0.7
1 week	98.0	0.8
2 weeks	96.7	1.1
3 weeks	96.6	1.3

Table 2. Stability data of [^{14}C]-MLN9708 at low specific activity (stored at −80 °C)

Time point	Radiochemical purity, %	Largest individual radiochemical impurity, %
Initial	99.4	0.2
7 weeks	99.0	0.3
11 weeks	98.9	0.3
14 weeks	98.1	0.5

Table 3. Stability data of [^{14}C]-2,5-dichlorohippuric acid at high specific activity (stored at −80 °C)

Time point	Radiochemical purity, %	Largest individual radiochemical impurity, %
Initial	99.7	0.2
1 month	99.3	0.3
2 months	98.4	0.7
3 months	98.1	0.7



Scheme 2. Synthesis of [^{14}C]-MLN9708 (19).

that the drug substance of lower specific activity and the dichlorohippuric acid precursor are adequately protected when stored at −80 °C for up to 3 months.

Experimental

General

All commercial reagents and solvents were used as supplied unless otherwise noted. [^{13}C]-labeled intermediates were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA) [^{14}C]-Barium carbonate was purchased from American Radiolabeled Chemicals, Inc. (Saint Louis, MO, USA)^{1}H-NMR spectra were recorded on a Varian is part of Agilent Technologies now (Santa Clara, CA, USA) 300 MHz and a Bruker (Billerica, MA, USA) 500 MHz. Radioactivity was quantified by liquid scintillation counting using Beckman (Brea, CA, USA) LS6500 counter. Purities and stability data for [$^{13}C_9$]-MLN9708 and [^{14}C]-MLN9708 were determined by HPLC (Agilent (Santa Clara, CA, USA) 1100) using Phenomenex Luna C8(2) 4.6 × 150 mm, 3-μm column, using the solvent combination of A (water, 0.1% formic acid) and B (acetonitrile, 0.1% formic acid) under gradient conditions: time 0 min 15% B; time 30 min 45% B; time 35–40 min 70% B; time 41–45 min 15% B. The purity and stability data for [^{14}C]-2,5-dichlorohippuric acid were evaluated by HPLC (Agilent 1100) using Phenomenex Luna C18(2) 4.6 × 150 mm, 5-μm column, using the solvent combination of A (water, 0.1% formic acid) and B (acetonitrile, 0.1% formic acid) under gradient conditions from 5% to 95% B in 15 min. Radioactive detection employed a LabLogic β-ram model 4 flow detector using Ultima-Flo M is a Perkin-Elmer product (Waltham, MA, USA) scintillant at 3 mL/min. MS determinations were performed on Agilent and Thermo LCQ (Waltham, MA, USA) mass spectrometers.

*[$^{13}C_7$]-2′,5′-Dichloroacetophenone (**4**)*

A three-neck round bottom flask equipped with thermocouple and reflux condenser topped with a calcium chloride drying tube was charged with [$^{13}C_6$]-1,4-dichlorobenzene (**3**, 2 g, 13 mmol) and anhydrous aluminum trichloride (4.1 g, 31 mmol, 2.3 eq) under an atmosphere of nitrogen. The reaction mixture was warmed to 47–50 °C and was stirred. To the mixture was added [1-^{13}C]-acetyl chloride (1.5 mL, 21 mmol, 1.6 eq) dropwise via syringe while maintaining an internal temperature of 60–65 °C. A suspension formed, and when the temperature was raised to 100 °C, a solution was formed. It was stirred at that temperature for 5 h. When the reaction was completed as determined by HPLC (using Phenomenex Luna C18(2) 4.6 × 150 mm, 5 μm column, with the solvent combination of A (water, 0.1% formic acid) and B (acetonitrile, 0.1% formic acid) under gradient conditions from 5% to 95% B in 15 min) and TLC (10% ethyl acetate in hexane), it was quenched by slowly pouring into 100-mL ice-water mixture with vigorous stirring (HCl is given off). The product was extracted with two portions of dichloromethane, and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ (100 mL) and water (100 mL). The dichloromethane solution was dried over $MgSO_4$, filtered and concentrated down to a brown residue. The desired compound was isolated by column chromatography using a gradient of 10% ethyl acetate in hexane (1.19 g, 40% and 90% purity).

^{1}H-NMR (DMSO): δ 8.04–8.12 (0.5H, m), 7.78–7.92 (1H, m), 7.50–7.57 (0.5H, m), 7.20–7.40 (1H, m), 2.57–2.61 (3H, dd).

*[$^{13}C_7$]-2′,5′-Dichlorobenzoic acid (**5**)*

To a chilled mixture of 1N NaOH (62 mL, 62 mmol, 11 eq) and aqueous NaClO (6% available chlorine, 95 mL, 84 mmol, 16 eq) was added dropwise a solution of [$^{13}C_7$]-2′,5′-dichloroacetophenone (**4**, 1.18 g, 5.4 mmol, 1 eq, 90% purity) in 1-mL dioxane. The mixture turned first cloudy, then cleared out. It was left stirring at room temperature over 72 h. The reaction was quenched by adding sodium thiosulfate pentahydrate, followed by dichloromethane. The layers were separated, and the aqueous layer that contained the product was acidified to pH 3 by adding concentrated HCl dropwise. The mixture was cooled in ice bath, and the white solid formed was filtered, washed with ice-cold water and dried under high vacuum to obtain the title compound (1.33 g, 100%).

^{1}H-NMR (DMSO): δ 8.04–8.12 (0.5H, m), 7.78–7.92 (1H, m), 7.50–7.57 (0.5H, m), 7.20–7.40 (1H, m). MS: m/z 196 (M-1).

*[$^{13}C_7$]-2′,5′-Dichlorobenzoyl chloride (**6**)*

[$^{13}C_7$]-2′,5′-dichlorobenzoic acid (**5**, 1.3 g, 6.8 mmol) was suspended in thionyl chloride (20 mL, 300 mmol, 40 eq) under an atmosphere of nitrogen and warmed up to 50 °C for 2 h. A small sample was tested by quenching with methanol, and the formation of the methyl ester was monitored by LC–MS. As only half of the starting material was consumed, additional thionyl chloride (up to 30 mL) was added, and heating was continued. Upon completion, the reaction mixture was cooled, concentrated by rotary evaporation to an oil, and used as it is in the next step.

*[$^{13}C_9$]-[(2,5-Dichlorobenzoyl)amino]acetic acid (**7**)*

A solution of the product from the previous step in 1.5-mL tetrahydrofuran was added dropwise to a mixture of [$^{13}C_2$]-glycine (2 g, 20 mmol, 3 eq) in 2.5-M NaOH aqueous solution (17 mL, 43 mmol, 6.3 eq). The mixture was stirred overnight at room temperature. Upon completion of the reaction, the mixture was brought to neutral pH by adding 2N HCl dropwise, then to pH 4 by 6N HCl. A tan solid precipitated, it was cooled in ice bath for 1 h, then isolated by filtration (73% pure by HPLC at 254 nm). The purity of the solid was improved by crystallization from water (35 mL) at 100 °C. After cooling and filtration, a white solid was recovered with 99% purity by HPLC (551 mg, 32% over two steps).

^{1}H-NMR (DMSO): δ 12.60–12.77 (1H, br), 8.86–8.95 (1H, br), 7.70–7.95 (1.5H, m), 7.15–7.35 (1.5H, m), 4.11–4.18 (1H, q), 3.64–3.72 (1H, q). MS: m/z 255 (M-1).

*[$^{13}C_9$]-2,5-Dichloro-N-(2-(((R)-3-methyl-1-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl) butyl)amino)-2-oxoethyl)benzamide (**9**)*

A mixture of [$^{13}C_9$]-[(2,5-dichlorobenzoyl)amino]acetic acid (**7**, 550 mg, 2.1 mmol), (1*S*,2*S*,3*R*,5*S*)-pinanediol leucine boronate trifluoroacetate salt (**8**, 810 mg, 2.1 mmol, 1 eq) and TBTU (760 mg, 2.4 mmol, 1.1 eq) was stirred in anhydrous DMF at room temperature for 15 min under an atmosphere of nitrogen. The mixture was cooled in ice bath, and *N*,*N*-diisopropylethylamine (1.1 mL, 6.4 mmol, 3 eq) was added dropwise at a rate of 1 drop/30 s. The reaction was allowed to proceed at 0 °C for 1 h. Upon completion verification by LC–MS, the reaction was diluted with 10-mL ethyl acetate, followed by water (10 mL). After the layers were separated, the organic extract was further washed with water (2 × 10 mL). The ethyl acetate extract was then washed sequentially with 15 mL each of 2 wt% aqueous solution of K_2CO_3, 1 wt% aqueous solution of H_3PO_4 and 10 wt% aqueous solution of NaCl. The final organic extract was concentrated by rotary evaporation, then redissolved in heptanes (5 mL) and concentrated again to provide a yellow solid (933 mg, 88%).

^{1}H-NMR (DMSO): δ 8.82–8.88 (2H, m), 7.75–7.88 (1.5H, m), 7.20–7.38 (1.5H, m), 4.17–4.25 (1H, q), 4.05–4.15 (1H, dd), 3.70–3.78 (1H, q), 2.55–2.65 (1H, m), 2.15–2.25 (1H, m), 1.95–2.05 (1H, m), 1.57–1.85 (4H, m), 1.15–1.40 (6H, m), 0.70–0.85 (12H, m). MS: m/z 504 (M + 1).

*[$^{13}C_9$]-(R)-(1-(2-(2,5-Dichlorobenzamido)acetamido)-3-methylbutyl) boronic acid (**10**)*

To a well-stirred mixture of [$^{13}C_9$]-2,5-dichloro-*N*-(2-(((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2] dioxaborol-2-yl)butyl)amino)-2-oxoethyl)benzamide (**9**, 933 mg, 1.85 mmol) and (2-methylpropyl)boronic acid (470 mg, 4.6 mmol, 2.5 eq) in methanol (10 mL) and hexane (10 mL) under an atmosphere of nitrogen was added 1 N HCl (5.6 mL, 5.6 mmol, 3 eq) dropwise. The solution was left stirring overnight at room temperature. Upon completion of reaction by HPLC, the two layers were separated, and the methanol (bottom) layer was washed with additional heptane (10 mL). The methanol layer was concentrated, redissolved in 10 mL of 2 N NaOH and washed with dichloromethane (3 × 10 mL). The aqueous layer was made acidic (pH 5) by adding 2 N HCl dropwise, then the product was extracted into dichloromethane (5 × 10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated down to a small volume of solvent.

Upon adding heptanes (3 mL), a white solid was formed. After filtration, it was dried over night under high vacuum to obtain the title compound (400 mg, 58%).

^{1}H-NMR (DMSO): δ 8.93–9.02 (1H, br), 8.68–8.80 (1H, br), 7.75–8.00 (1.5H, m), 7.20–7.50 (1.5H, m), 4.20–4.30 (1H, t), 3.75–3.95 (1H, t), 2.60–2.70 (1H, m), 1.57–1.70 (1H, m), 1.15–1.40 (2H, m), 0.70–0.85 (6H, m). MS: m/z 352 (M + 1-H_2O).

*[$^{13}C_9$]-(R)-2,2′-(2-(1-(2-(2,5-Dichlorobenzamido)acetamido)-3-methylbutyl)-5-oxo-1,3,2-dioxaborolane-4,4-diyl)diacetic acid (**11**)*

A solution of citric acid (128 mg, 0.6 mmol, 1.1 eq) in ethyl acetate (2.5 mL) was prepared by heating at 60 °C under an atmosphere of nitrogen. Separately, a similar solution of [$^{13}C_9$]-(*R*)-(1-(2-(2,5-dichlorobenzamido) acetamido)-3-methylbutyl)boronic acid (**9**, 222 mg, 0.6 mmol) was prepared in ethyl acetate (0.5 mL). It was added dropwise to the hot citric acid solution. After stirring at 60 °C for 15 min, the solution was cooled slowly to room temperature, when a cloudy precipitate was formed. Further cooling in ice bath and filtering followed by drying under high vacuum at 40 °C provided the desired compound (287 mg, 91%).

^{1}H-NMR (DMSO): δ 12.10–12.40 (1H, br), 10.65–10.90 (1H, br), 9.10–9.30 (1H, br), 8.99–7.75 (1.5H, m), 7.20–7.50 (1.5H, m), 4.45–4.60 (1H, t), 3.95–4.15 (1H, t), 2.85–2.95 (1H, t), 2.60–2.80 (4H, m), 1.57–1.70 (1H, m), 1.15–1.40 (2H, m), 0.70–0.85 (6H, m).

*[^{14}C]-2,5-Dichlorobenzoic acid (**14**)*

To a solution of 1-bromo-2,5-dichlorobenzene (**12**, 580 mg, 2.5 mmol) in ether (6 mL) at −78 °C and under an atmosphere of nitrogen was added 1.6-M *n*-butyl lithium in hexanes (1.6 mL, 1 eq) dropwise. The resulting yellow solution was stirred at low temperature for 30 min. This solution containing lithiated species **13** was treated at −78 °C with a freshly prepared stream of $^{14}CO_2$ (generated from 100 mCi of $Ba^{14}CO_3$, 58.4 mCi/mmol, and concentrated H_2SO_4) for 4 h. The reaction was quenched by adding water slowly (6 mL) and 5% aq. Na_2CO_3 (1 mL). The layers were separated, and the organic layer was further washed with 5% aq. Na_2CO_3 (4 mL). The combined basic extracts were neutralized with concentrated HCl (1 mL), and the product was extracted into dichloromethane (3 × 8 mL). After drying over $MgSO_4$ and filtering, the organic solution was concentrated to a white solid, which was dried under high vacuum to give the title compound (300 mg, 62%).

*[^{14}C]-Methyl-[(2,5-dichlorobenzoyl)amino]acetate (**15**)*

A mixture of [^{14}C]-2,5-dichlorobenzoic acid (**14**, 300 mg, 1.57 mmol), glycine methyl ester (197 mg, 1.5 mmol, 1 eq), 1-hydroxybenzotriazole (210 mg, 1.6 mmol, 1 eq) and *N*,*N*-diisopropylethylamine (0.55 mL, 3.2 mmol, 2 eq) was dissolved in tetrahydrofuran (7 mL) under an atmosphere of nitrogen. The solution was cooled in ice bath and finally added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (300 mg, 1.5 mmol, 1 eq). The resulting yellow solution was stirred at room temperature overnight. The reaction was quenched by adding saturated aq. $NaHCO_3$ solution (8 mL), and the product was extracted with dichloromethane (3 × 10 mL). The organic extracts were concentrated to an orange oil and used as it is in the next step (410 mg, 87%).

*[^{14}C]-[(2,5-Dichlorobenzoyl)amino]acetic acid (**16**)*

To a solution of [^{14}C]-methyl-[(2,5-dichlorobenzoyl)amino]acetate (**15**, 410 mg, 1.55 mmol) in methanol (5 mL) was added lithium hydroxide (130 mg, 3.1 mmol, 2 eq) and water (1.5 mL). The reaction was stirred at room temperature overnight. It was acidified with 6 N HCl (1 mL), and the organic solvent was removed by rotary evaporation. The remaining mixture was partitioned between water (5 mL) and ethyl acetate (10 mL). After the layers were separated, the aqueous extract was further washed with ethyl acetate (2 × 10 mL). The combined organic extracts were concentrated down to a yellow oil, which was recrystallized from hot water (5 mL). After cooling to room temperature, a white solid of high radiochemical purity was isolated by filtration (92 mg, 23%).

*[^{14}C]-[(2,5-Dichloro-N-[2-({(1R)-3-methyl-1-[(3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methano-1,3,2-benzodioaxaborol-2-yl] butyl}amino)-2-oxoethyl}benzamide (**17**).*

A mixture of [^{14}C]-[(2,5-dichlorobenzoyl)amino]acetic acid (**16**, 91 mg, 0.36 mmol), (1*S*,2*S*,3*R*,5*S*)-pinanediol leucine boronate trifluoroacetate salt (**8**, 140 mg, 0.37 mmol, 1 eq) and TBTU (130 mg, 0.4 mmol, 1.1 eq) was stirred in anhydrous DMF (2 mL) at room temperature for 15 min under an atmosphere of nitrogen. This mixture was cooled in ice bath, and *N*,*N*-diisopropylethylamine (0.2 mL, 1 mmol, 3 eq) was added dropwise at a rate of 1 drop/30 s. The reaction was allowed to proceed at 0 °C for 1 h and quenched with water (5 mL). A chunky white solid was formed, and after stirring at room temperature for 30 min, it was filtered and dried under high vacuum (165 mg, 90%).

*[^{14}C]-(R)-(1-(2-(2,5-Dichlorobenzamido)acetamido)-3-methylbutyl) boronic acid (**18**)*

A mixture of [^{14}C]-[(2,5-dichloro-*N*-[2-({(1*R*)-3-methyl-1-[(3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methano-1,3,2-benzodioaxaborol-2-yl]butyl} amino)-2-oxoethyl}benzamide (**17**, 165 mg, 0.33 mmol), (2-methylpropyl) boronic acid (80 mg, 0.8 mmol, 2 eq) in methanol (10 mL) and hexane (10 mL) with 1 N HCl (1 mL, 1 mmol, 3 eq) was stirred at room temperature for 4 h. Upon completion of reaction by HPLC, the two layers were separated, and the methanol (bottom) layer was washed with additional hexane (10 mL). The methanol layer was concentrated, redissolved in 2 mL of 2 N NaOH and washed with dichloromethane (3 × 5 mL). The aqueous layer was made acidic (pH 5) by adding 1 N HCl dropwise, then the product was extracted into dichloromethane (5 × 10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated down. The residue was redissolved in dichloromethane, and upon addition of hexane (1.5 mL), a white solid formed. Solvent was removed under a stream of nitrogen to obtain the title compound (79 mg, 91% radiochemical purity). The impure product was redissolved in a mixture of water, and acetonitrile and chromatographed on a Phenomenex Luna C8(2) column (10 × 250 mm, 5 μm, flow rate: 4 mL/min, 234 nm, linear gradient elution from15% B to 45% B in 40 min, ramp up to 45% B in 5 min, then 95% B in 5 min). Radiochemical purity of the combined fractions was 99% (60 mg, 50%).

MS: m/z 345 (M + 1-H_2O).

*[^{14}C]-(R)-2,2′-(2-(1-(2-(2,5-Dichlorobenzamido)acetamido)-3-methylbutyl)-5-oxo-1,3,2-dioxaborolane-4,4-diyl)diacetic acid (**19**)*

A solution of citric acid (33 mg, 0.17 mmol, 1 eq) in ethyl acetate (1.5 mL) was prepared by heating at 60 °C under an atmosphere of nitrogen. Separately was prepared a similar solution of [^{14}C]-(*R*)-(1-(2-(2,5-dichlorobenzamido) acetamido)-3-methylbutyl)boronic acid, (**18**, 60 mg, 0.17 mmol) in ethyl acetate (0.5 mL). It was added dropwise to the hot citric acid solution. After stirring at 60 °C for 10 min, the solution was cooled slowly to room temperature. It became cloudy as a precipitate formed and further cooled in ice bath, then filtered and dried under high vacuum at 40 °C to obtain the desired compound (79 mg, 7.3 mCi, 90%, 108.8 μCi/mg).

^{1}H-NMR (DMSO): δ 12.0–12.20 (1H, br), 10.63–10.85 (1H, br), 9.10–9.25 (1H, br), 7.79–7.75 (1.H, s), 7.70–7.58 (2H, s), 4.43–4.22 (1H, t), 3.95–4.15 (1H, t), 2.85–3.2.95 (1H, t), 2.60–2.80 (4H, m), 1.84–1.67 (1H, m), 1.45–1.20 (2H, m), 0.78–1.00 (6H, m).

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Conflict of interest

The authors did not report any conflict of interest.

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